Comparing naturally occurring stable isotopes of nitrogen, carbon, and strontium as markers for the rearing locations of Atlantic salmon (*Salmo salar*)

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Abstract: We compared the success of using naturally occurring stable isotopes of N, C, and Sr as markers for the rearing locations of juvenile salmon. We analyzed the isotopic signatures ($\delta^{15}N$ and $\delta^{13}C$ in muscle and scales and $^{87}Sr/^{86}Sr$ in otoliths) of >200 juvenile Atlantic salmon (*Salmo salar*) from 12 tributaries of the Connecticut River, USA. Young salmon had distinct N and C signatures 5 weeks after stocking. Signatures were stable over the summer although $\delta^{13}C$ varied more than $\delta^{15}N$ or $^{87}Sr/^{86}Sr$. Scale and muscle signatures were highly correlated, demonstrating the feasibility of nonlethal sampling using fish scales. Some C (but not N) signature from the hatchery was retained in scales of 3-month-old fish, implicating scale annuli as a repository for past C signatures. The $\delta^{15}N$ values successfully differentiated fish from tributaries with differences in land use (e.g., agricultural versus forested; ≈33% of sites); the $\delta^{13}C$ values differentiated fish from 45% of sites. Based upon a discriminant-function analysis, group membership of individuals was correctly predicted in 44.3% (74 of 167) of cases for which both N and C were analyzed. In combination, N and C isotopes differentiated 73% of study sites, which was close to the success of Sr isotopes in the same system (83%).

Résumé : Nous avons comparé la performance des isotopes stables de N, C et Sr qui existent en nature comme marqueurs du lieu où se sont développés des jeunes saumons. Nous avons analysé les signatures isotopiques ($\delta^{15}N$ et $\delta^{13}C$ dans le muscle et les écailles et $^{87}Sr/^{86}Sr$ dans les otolithes) chez >200 jeunes saumons atlantiques (*Salmo salar*) dans 12 tributaires du fleuve Connecticut, États-Unis. Les jeunes saumons possèdent des signatures N et C distinctes 5 semaines après l'empoissonnement. Les signatures demeurent stables au cours de l'été bien que $\delta^{13}C$ varie plus que $\delta^{15}N$ ou que $^{87}Sr/^{86}Sr$. Il y a une forte corrélation entre les signatures du muscle et des écailles; il est donc possible de faire des prélèvements non létaux au moyen des écailles des poissons. Des vestiges de la signature de C (mais non de N) de la pisciculture persistent dans les écailles des poissons âgés de 3 mois; les annulus des écailles sont donc des points de rétentions des signatures antérieures de C. Les valeurs de $\delta^{15}N$ permettent de séparer avec succès des tributaires dans des zones d'utilisation des terres différentes (par exemple, les zones agricoles des zones forestières : ≈33 % des sites); $\delta^{13}C$ permet la reconnaissance de 45 % des sites. Une analyse des fonctions discriminantes permet de prédire correctement l'appartenance des individus à un groupe dans 44,3 % (74/167) des cas lorsque C et N ont été tous deux analysés. La combinaison des isotopes N et C permet de séparer 73 % des sites, ce qui s'approche de la performance des isotopes Sr dans le même système (83 %).

[Traduit par la Rédaction]

Introduction

Geochemical signatures are increasingly used to identify geographic sources, habitat use, and movements of animals (Campana and Gagne 1995; Kennedy et al. 1997; Thorrold et al. 1998). Animals preserve chemical records from their environment in their tissues. As a result, in aquatic systems predictable relationships arise between underlying geology, land use, water chemistry, food-web structure, and tissue chemistry of residents of particular habitats (Limburg 1995;

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Harrington et al. 1998; Thorrold et al. 2001). Efforts to link fish to specific geographic locations and to supplement conventional fish-tagging techniques using unique geochemical markers have been compelling and productive.

Stable isotopes offer numerous advantages over other stock-identification techniques for identifying rearing locations (for a review see Hobson 1999). Isotopic signatures occur naturally and therefore require no invasive handling or application cost. Techniques for measuring isotope ratios are precise and steadily improving. Isotope ratios can be measured in fish of all ages and sizes and so are especially useful in larval fish, which are too small for conventional tags. Finally, isotopic signatures in fish (France 1995*b*; Kennedy et al. 1997; Wells et al. 2000*b*) and other animals (Marra et al. 1998; Blum et al. 2001; Rubenstein et al. 2002) correlate highly with environmental conditions in their rearing habitats.

Isotopic signatures that originate in underlying bedrock (e.g., Sr isotopes) are particularly useful because they remain fairly constant seasonally and annually (Kennedy et al. 2000; Peters et al. 2004). Consequently, Sr isotope signatures are stable as long as a fish remains in a particular site. Yet no single isotope can discriminate among an infinite number of source locations, so the isotopic signatures of multiple elements are often used to greatly enhance discriminatory power (Koch et al. 1995; Doucett et al. 1999). In any particular study, the choice of suitable elements or isotopes for providing habitat signatures will depend upon considerations of both precision and price.

Many different elemental (e.g., Mg, Ba, Sr, Mn relative to Ca) and isotope ratios (e.g., $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $^{87}Sr/^{86}Sr$) have been used to trace the origins of moving and migrating fish (Doucett et al. 1999; Hobson 1999; Thorrold et al. 2001). Sr has been among the most successful because of its relative abundance in nature and because it provides both an isotopic and an elemental fingerprint with high precision (Kennedy et al. 2000, 2002). Sr is incorporated into the growing tissue of fish through substitution for Ca. Sr signatures in their calcified tissues mirror Sr signatures in the water in which they are reared (Kennedy et al. 2000). Large differences between freshwater and marine Sr concentrations enable the separation of anadromous from non-anadromous fish stocks based upon the elemental ratio of Sr to Ca in their bones and otoliths (Limburg 1995; Friedland et al. 1998; Radtke et al. 1998). In comparison, stable isotope signatures of Sr in fish reflect differences in the $^{87}Sr/^{86}Sr$ ratios that arise from relatively small differences in watershed geology (Kennedy et al. 1997; Ingram and Weber 1999; Kennedy et al. 2000). Thus, they can be used to distinguish fish habitat use over comparatively small spatial scales. A close match between the isotopic signature of a fish and that in a particular stream indicates that the fish could have reared in that habitat (Kennedy et al. 2002). Discrepancies between the isotopic signature of individuals and that of their stream-water indicate that there has been movement among streams (Kennedy et al. 2000, 2002). Sr isotopes have made it possible to distinguish fish from multiple tributaries within a catchment (Kennedy et al. 1997; Ingram and Weber 1999) and to resolve the movements of individual fish among rearing streams (Kennedy et al. 2000, 2002). This level of spatial resolution in freshwater systems (1–10 km) has not been possible using elemental ratios (e.g., Sr/Ca).

In this study, we quantitatively compare the relative success of naturally occurring stable isotopes of N, C, and Sr at distinguishing among rearing tributaries of juvenile Atlantic salmon (*Salmo salar*). A comparison of these isotopic systems is useful because most laboratories will have the capacity to measure some isotopic ratios but not others. Moreover, these isotopic systems each mark different aspects of the stream habitat and may vary over different temporal and spatial scales. For example, the $\delta^{15}N$ value of stream water is sensitive to differences in land-use patterns at the watershed scale because many agricultural processes result in enrichment of $\delta^{15}N$ relative to forested areas (McClelland et al. 1997; Harrington et al. 1998; Lake et al. 2001). The $\delta^{15}N$ value is also used to determine the trophic positions of fish and other animals (Deniro and Epstein 1978; Fry 1991; Cabana and Rasmussen 1994). In comparison, the $\delta^{13}C$ value of stream organisms varies seasonally and spatially as a function of the source of organic matter (e.g., benthic algal versus terrestrial C subsidies) or the longitudinal location within a watershed (Rounick and Winterbourn 1986; Finlay et al. 1999; Finlay 2001). Finally, the $^{87}Sr/^{86}Sr$ ratio of stream water is tightly linked to the underlying watershed geology (Kennedy et al. 2000), which is very stable over time. Sr isotopes also do not fractionate significantly within organisms, and any fractionation that might occur is removed from the isotopic signal during analysis (Kennedy et al. 2000). Hence, these isotopes investigated together are likely to provide a more powerful tool for stock discrimination than each does alone. Other researchers have investigated the use of isotopes or trace elements in combination to follow movements or origins of various species of fish (Hansson et al. 1997; Thorrold et al. 1998; Doucett et al. 1999), birds (Marra et al. 1998; Rubenstein et al. 2002), or invertebrates (DiBacco and Levin 2000). However, none compare the stable-isotope ratios of these three key elements or evaluate them at the spatial resolution of our study.

We measured the isotopic signatures ($\delta^{15}N$, $\delta^{13}C$) in muscle tissue and scales of >200 juvenile (age 0) Atlantic salmon stocked into 12 tributaries of the Connecticut River, USA, as part of the Atlantic salmon restoration program. We first compare the ability of $\delta^{15}N$ and $\delta^{13}C$, alone and in combination, to distinguish fish from different sites. We then compare $\delta^{15}N$ and $\delta^{13}C$ signatures relative to the power of $^{87}Sr/^{86}Sr$ ratios in fish from the same region, based upon our previous analyses (Kennedy et al. 2000). Specifically, we quantify (*i*) the rates at which N- and C-isotope signatures are incorporated into salmon fry after stocking, (*ii*) the stability of isotopic signatures in fish tissues within the first summer of growth, (*iii*) the relationships between the N- and C-isotope signatures in scales and muscle tissue (with particular interest in the feasibility of developing a method for nonlethal isotope assessment), and (*iv*) the ability of $\delta^{13}C$ and $\delta^{15}N$ to distinguish between fish from different sites and how the discriminatory power of these two isotopes compares with that of a geologically derived tracer, the $^{87}Sr/^{86}Sr$ ratio.

Methods

We collected juvenile Atlantic salmon from 12 stream tributaries of the West and White rivers in central and south-

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ern Vermont, USA (Fig. 1), for C- and N-isotope analysis. These streams are stocked annually in early May with unfed hatchery-raised fry (<30 mm). Currently 15% of the more than 10 million fry stocked annually in the Connecticut River restoration program are placed in the tributaries of the West and White rivers. We sampled the muscle tissue and scales of 200 juvenile salmon from four tributaries in the West River basin and eight sites in the White River basin in 1991, 1995, and 1996. We analyzed and previously reported $^{87}Sr/^{86}Sr$ ratios in salmon collected from West River and White River tributaries (Kennedy et al. 1997, 2000) and some of these results are included to compare the efficacy of discriminating populations based upon stable isotopes of N, C, and Sr separately and in combination.

Sample collection and preparation

We collected underyearling Atlantic salmon by means of a combination of electroshocking and dip-netting techniques (described in Nislow et al. 1999). Wet weights and lengths were taken in the field. Fish were immediately placed on ice and stored frozen at –20 °C. Prior to isotopic analysis, samples were thawed and dissected in the laboratory. Dissections were cleanly performed using acid-washed Teflon® forceps or clean stainless-steel tools. For consistency, a small sample of bone-free muscle tissue was taken from the left side of each fish posterior to the dorsal fin. Between 8 and 10 scales were removed from the front left side of the fish dorsal to the lateral line. Scales were first rinsed thoroughly with triple-deionized and distilled (3×) water, then any visible organic residue was carefully scraped off under a dissecting microscope. Scales were then rinsed with ultrapure dilute (0.1 mol·L^{-1}) NaOH to remove any additional residue, ultrasonically cleaned for 5 min in 3× water, and rinsed thoroughly with 3× water. Muscle tissue was rinsed thoroughly with 3× water. Muscle tissue and scales were dried overnight at 60 °C and ground to a fine powder in a Wiley Mill with a No. 40 sieve and subsamples were weighed in tin capsules. To retrieve enough material, multiple scales from individuals were combined for each single analysis.

Uptake and stability of N and C isotopes

To measure the rate of incorporation and temporal variability of N- and C-isotope signatures in salmon muscle tissue, we sampled juvenile salmon in six sites (Marlboro, Flood, Utley, Bethel Gilead, Tweed, and Hancock) up to three times during their first growing season in 1991. These periods corresponded to approximately 40–50, 60–75, and 80–107 days after stocking. Between 5 and 10 individuals were caught and analyzed for a given site and date. Hatchery fry, which were obtained prior to stocking in 1995 and 1996 from the White River National Fish Hatchery in Bethel, Vermont, USA, were pooled and used to give an initial isotopic signature. All tissues were prepared for N- and C-isotope analysis as described under "Analytical procedures".

Relationship between $\delta^{15}N$ and $\delta^{13}C$ signatures of salmon muscle tissue and scales

To compare isotopic signatures of different fish tissues, we compared $\delta^{15}N$ and $\delta^{13}C$ in scales and muscle tissue from salmon collected in six sites (5–8 fish per site) 4 months after stocking (in 1996). The six sites (White River – Peavine, First Branch, Third Branch, West Branch, Bingo Brook, and Bethel Gilead) were selected to span the land-use patterns that typify the relatively forested headwater streams in the region (see Harrington et al. 1998) and thus to produce a potential range of $\delta^{15}N$ and $\delta^{13}C$ signatures. Salmon from all sites were pooled in the analysis. We analyzed different individuals from these six sites previously for N in muscle tissue only (Harrington et al. 1998). However, a completely new data set for C and N in muscle tissue and scales is presented here.

Distinguishing among salmon populations

To characterize regional variation in $\delta^{15}N$ and $\delta^{13}C$ signatures across salmon streams, we tested for differences in isotopic signatures among fish from 12 study streams (11 sites for $\delta^{13}C$ because fish from 1 site were only analyzed for N). These are the streams referred to in the previous sections that were used to test for uptake, stability, and the relationship between signals in muscle and scales (Fig. 1). All fish taken from a site were used to establish a mean signature for each site. In cases where fish were collected at different times throughout the growing season, data were pooled over all sampling dates. We felt that pooling across the entire time period would ensure proper interspersion of samples across the growing season and the most conservative test of site differences, because variability in the isotopic signatures is larger when data are included for the entire time period than for any single time point. To test the effect of pooling all individuals over the entire growing season on our ability to discriminate sites, we compared the results of two discriminant-function analyses for late-season fish from the five sites for which three sampling dates are represented. First, we attempted to reclassify the 37 late-season fish based upon discriminant functions constructed when all 118 fish were included and then compared the results with discriminant functions based only on those 37 fish.

We compared the mean $\delta^{15}N$ and $\delta^{13}C$ values for fish across sites using one-way analysis of variance. Analysis of single variables was performed using one-way analysis of variance followed by Fisher's least significant difference multiple comparisons test. Fisher's least significant difference multiple comparison ($p < 0.05$) was used to test for significant differences in $\delta^{15}N$ and $\delta^{13}C$ signatures across sites. Sites were placed in distinct groupings based upon whether they had significantly different C- and (or) N-isotope signatures. We performed multivariate analysis on $\delta^{15}N$ and $\delta^{13}C$ signatures using Fisher's linear discriminant function analysis to compare our among-site comparisons with individual reclassification rates. SPSS® for Windows (version 11.5.0; SPSS Inc. 2002) was used for the statistical analysis.

Analytical procedures

N- and C-isotope compositions were determined using an online Carlo Erba elemental analyzer instrument interfaced with a continuous-flow Finnigan MAT 252 (Thermo Finnigan MAT, Bremen, Germany) mass spectrometer located at Dartmouth College, New Hampshire, USA. Isotope ratios for N and C are presented as δ values, where $\delta = [(R_{sample}/R_{standard}) - 1] \times 1000$ and $R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$. The N- and C-isotope reference standards are air (N) and

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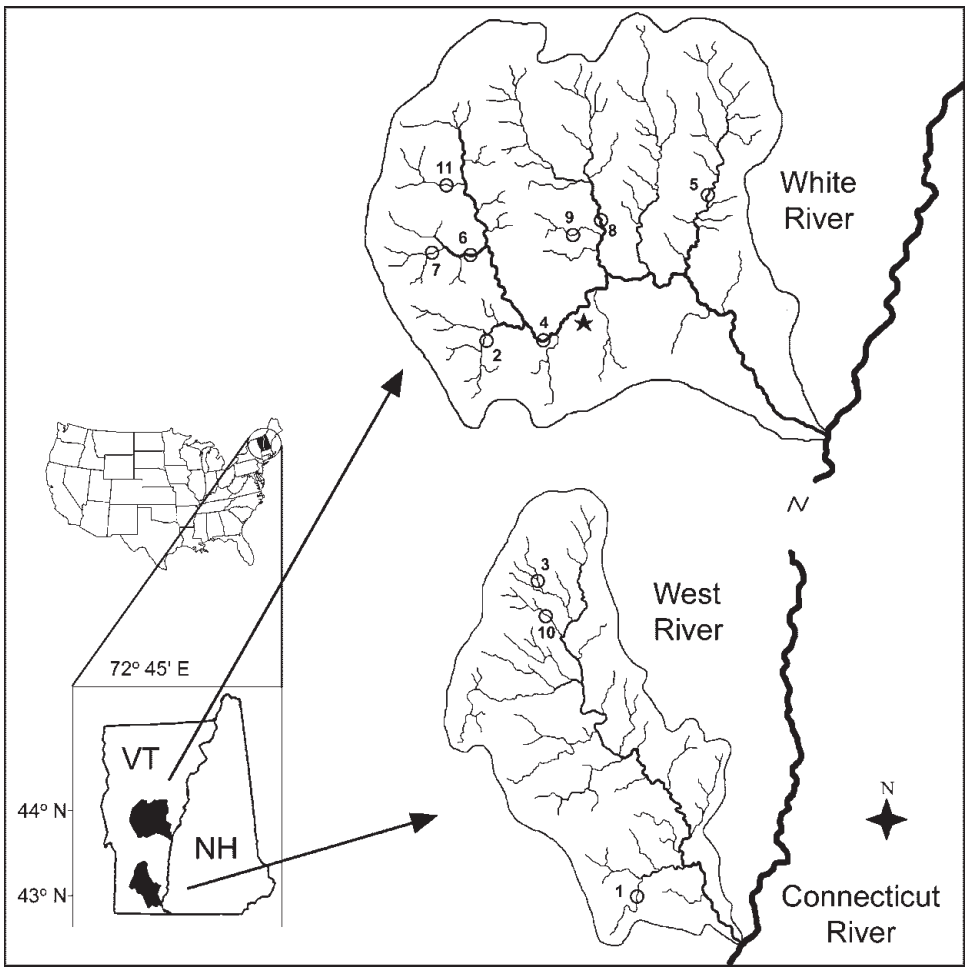
Fig. 1. Map of the study sites in the White and West rivers, Vermont, USA. The star beside the White River indicates the location of the White River National Fish Hatchery, which provides most of the Atlantic salmon (*Salmo salar*) fry for the stocking program. Numbered symbols identify study sites listed in Table 2 and shown in Fig. 4.



Peedee belemnite (C). Reported uncertainties are the standard error (SE) of the mean.

For each batch of analyses (approximately 10 samples) we prepared and analyzed our laboratory standard $(NH_4)_2SO_4$ (Baker Analyzed, VWR). The laboratory standard had a $\delta^{15}N$ value of –1.20 ± 0.02‰ ((mean ± 1 SE; n = 49) for the duration of this experiment. We also analyzed N standards with $\delta^{15}N$ values determined by the Geophysical Laboratory of the Carnegie Institution. We determined a value of 7.19 ± 0.06‰ (mean ± 1 SE) for the Geophysical Laboratory's $NaNO_3$ standard, which has a value of 7.2‰ (n = 8). For gelatin with a Geophysical Laboratory value of 7.4‰ for $\delta^{15}N$, we measured a value of 7.41 ± 0.07‰ (mean ± 1 SE) for five analyses. Overall, external precision on laboratory standards analyzed during the course of this experiment was better than ±0.15‰ for both $\delta^{15}N$ and $\delta^{13}C$.

Isotope analyses for Sr in fish vertebrae and otoliths and stream water from each site have been published (Kennedy et al. 1997, 2000). Briefly, Sr isotopic compositions were measured using either the VG-Sector or Finnegan MAT 262 thermal ionization mass spectrometers at Dartmouth College. Reported uncertainties are two times the standard error (2 × SE) calculated from the measurement of 100–300 ratios on each individual sample. The $^{87}Sr/^{86}Sr$ ratios were normalized to $^{88}Sr/^{86}Sr$ = 0.1194. Replicate analysis of a National Institute of Standards and Technology standard reference material (SRM-987), using the Finnegan MAT 262 and the VG-Sector, completed during the course of this study yielded mean $^{87}Sr/^{86}Sr$ ratios of 0.710262 (standard deviation (SD) = 0.000013, n = 32) and 0.710215 (SD = 0.000062, n = 12), respectively.

Results

Uptake and stability of N, C, and Sr isotopes

Incorporation of stream-specific $\delta^{15}N$ and $\delta^{13}C$ values into fish tissue occurred within the first 5 weeks after stocking (Table 1). The isotopic signature that developed within each site was relatively constant between 40 and 107 days after stocking (Fig. 2). Seasonal variability in the $\delta^{13}C$ signature was greater than that in $\delta^{15}N$, especially within the first 2 months. After 60 days the signal was more constant and the signatures of fish at each site remained distinct despite some within-site variation. Reclassification rates for late-season individuals did not change significantly regardless of whether the discriminant functions were constructed using N and C only from late-season individuals (23 of 37 or 62.2% correct) or from all individuals throughout the growing sea-

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Table 1. Stable-isotope values in salmon (*Salmo salar*) from the hatchery and collected from the field sites between 40 and 100+ days after stocking.

Site	Fish age (days)	$\delta^{15}N$	$\delta^{13}C$	*n*
Hatchery	0	14.48 (0.79)	–19.84 (0.49)	7
Flood	40	7.92 (0.20)	–21.16 (0.19)	3
	104	9.23 (0.22)	–22.46 (0.44)	9
Marlboro	40	8.71 (0.41)	–20.04 (0.57)	5
	66	8.79 (0.58)	–19.16 (1.22)	6
	93	9.24 (0.20)	–18.88 (0.78)	8
Utley	45	8.72 (0.33)	–23.47 (1.21)	7
	57	8.02 (0.18)	–23.47 (1.04)	10
	104	8.85 (0.30)	–24.89 (0.21)	2
Bethel Gilead	44	8.47 (0.68)	–21.79 (1.18)	9
	66	8.89 (0.45)	–22.21 (0.87)	9
	107	8.84 (0.58)	–22.78 (0.39)	8
Hancock	47	7.75 (0.51)	–21.56 (1.21)	9
	61	8.26 (0.51)	–21.78 (0.73)	9
	79	8.73 (0.61)	–23.74 (1.11)	10
Tweed	49	8.20 (0.49)	–25.26 (1.68)	9
	75	7.35 (0.12)	–22.83 (0.68)	5
	100	8.28 (0.58)	–23.54 (2.36)	9

Note: Values are given as the mean with the standard deviation in parentheses; *n* is the sample size.

son (20 of 37 or 54.1% correct). In comparison, when all 118 fish from these five sites were reclassified to site based upon discriminant functions, group membership was correctly predicted for 54.2% (64 of 118) of the cases. Kennedy et al. (2000) studied incorporation of stream-specific Sr isotope ratios into fish tissue and found that $^{87}Sr/^{86}Sr$ ratios in the vertebrae and otoliths of juvenile salmon in 29 stream sites in the West and White rivers also differed from the hatchery signal within the first 3 months after stocking and remained stable within a site over seasons and years.

Relationship between $\delta^{15}N$ and $\delta^{13}C$ values in salmon muscle tissue and scales

There was a strong positive correlation between the isotopic signatures in fish scales and muscle tissue (Fig. 3) for both $\delta^{15}N$ and $\delta^{13}C$ values. The dotted 1:1 line in the figures depicts complete equilibration between scales and muscle tissue. The deviation from the 1:1 line only observed for $\delta^{13}C$ (Fig. 3*b*) in muscle tissue and scales indicates that some of the $\delta^{13}C$ signal (but not the $\delta^{15}N$ signal) from the hatchery ($\delta^{13}C = -19.84 \pm 0.49$) was retained in the scales of 4-month-old fish. We showed previously that the $^{87}Sr/^{86}Sr$ signals in otoliths, scales, and backbones of juvenile Atlantic salmon were statistically indistinguishable (Kennedy et al. 2000).

Distinguishing among salmon populations

The different isotopic systems individually distinguished between juvenile salmon from different rearing locations to different extents. We determined this by testing to see how many clusters of distinct isotopic signatures could be identified among fish from the 11 sites for which we had both $\delta^{13}C$ and $\delta^{15}N$ values. Salmon from 3 of the 11 sites carried significantly different $\delta^{15}N$ signals than salmon from the other sites (Table 2; Fig. 4). These differences allowed us to discriminate among four separate groups (or 33%) of salmon across the study sites (Table 2). Differences in the $\delta^{13}C$ signals in fish distinguished between five separate groups (45%). When $\delta^{13}C$ and $\delta^{15}N$ data were combined, salmon from 7 of the 11 sites could be differentiated, permitting us to separate salmon across the region into eight (74%) isotopically distinct groups. Based upon a discriminant-function analysis, group membership of individuals was correctly predicted in 44.3% (74/167) of the cases in which both N and C were analyzed (Table 2). At the group level, the discriminatory power of $\delta^{15}N$ and $\delta^{13}C$ combined is close to that of Sr (83%), which allowed us to distinguish 8 of 8 tributaries in the White River and 7 of 10 tributaries in the West River (Kennedy et al. 2000) (Table 2). In most cases, N, C, and Sr analyses were not performed on exactly the same fish, therefore we were unable to determine the strength of combining all three isotopic systems in a single discriminant-function analysis.

Discussion

This study contributes to the rapidly growing literature identifying unique and valuable uses of stable isotopes as markers of animal movement and habitat use and food-web characteristics (Hansson et al. 1997; Thorrold et al. 2001; Kennedy et al. 2002). Our results demonstrate the feasibility and potential value of using light-stable isotopes (C and N) as markers for juvenile salmon rearing locations. We conducted these studies with hatchery alevins, which underscores the value of using chemical tracers to assess natal origin in fish that are too small to mark using more conventional methods. We also demonstrated that salmon isotopic compositions can be assessed nonlethally in their scales, which could be extremely important for adapting these techniques for use in endangered populations. Finally, two commonly measured isotopes (C and N) used together differentiated salmon from nearly as many locations as isotope ratios of Sr over the first season of growth. Site-specific differ-

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Fig. 2. Incorporation of (*a*) N and (*b*) C isotopic signatures 4 months after stocking in two representative study sites, Bethel Gilead (□) and Marlboro (●). Data exist for four other streams, but these two sites exemplify the general trends across all sites. Fry are stocked in May at weights of less than 0.20 g and weigh approximately 1.0–1.5 g at 40 days of age. By September, parr weigh between 4 and 8 g.

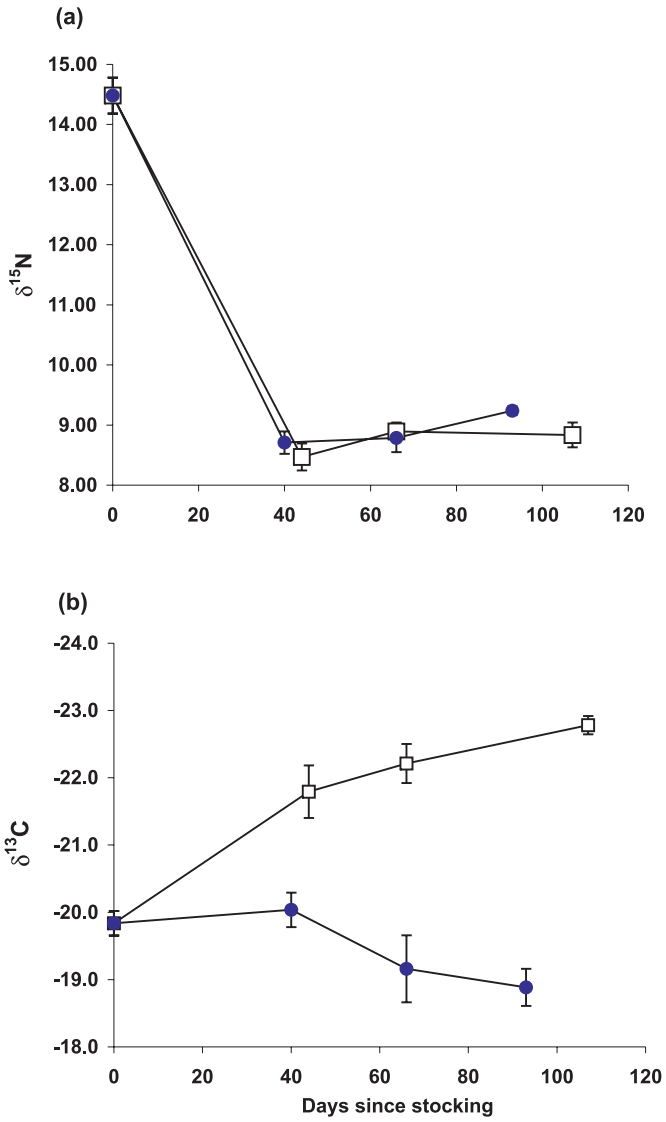
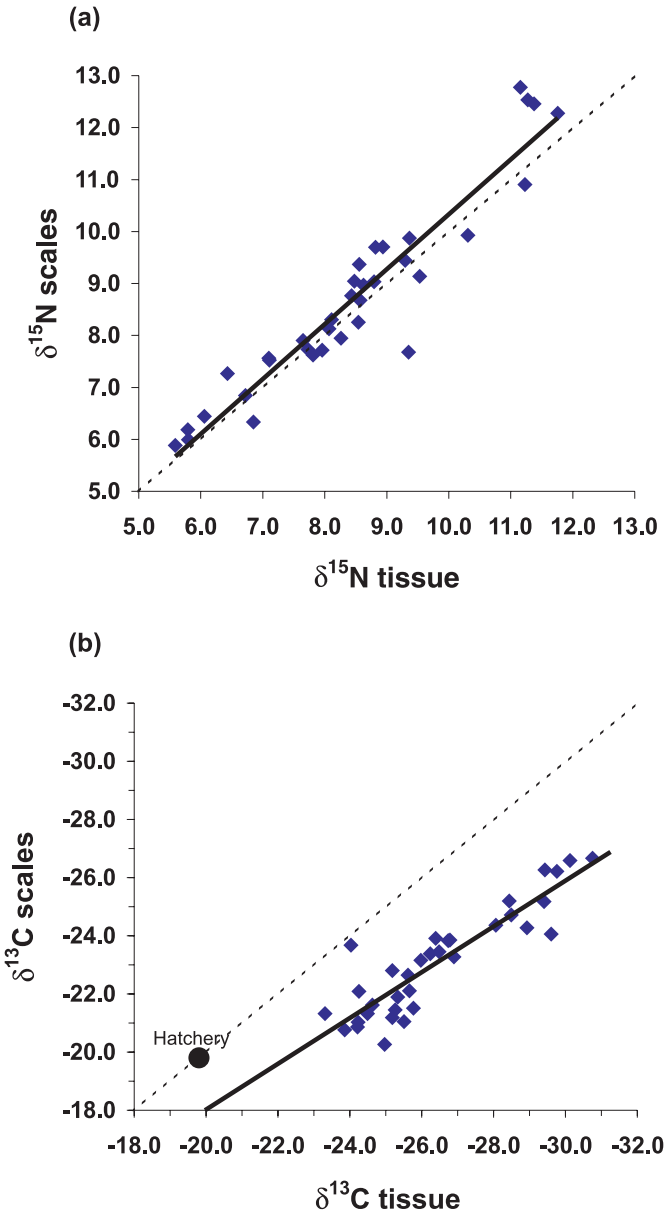


Fig. 3. (*a*) N and (*b*) C isotope ratios in age-0 Atlantic salmon (*Salmo salar*) tissue and scales. Each point represents an individual fish from one of six sites. The broken line represents a 1:1 relationship between $\delta^{15}N$ signatures in tissue and scales. There is a significant positive relationship between the isotopic signatures in muscle tissue and that of entire scales ($P < 0.001$). For N, $y = 1.06x - 0.23$, $r^2 = 0.90$, and for C, $y = 0.79x - 2.29$, $r^2 = 0.80$.



ences in these three isotopic systems that relate to land use, food-web structure, and geology will affect their relative suitability as markers of natal origin.

Uptake and stability

All three elements (N, C, and Sr) are incorporated quickly into the tissue of juvenile salmon. As a result of rapid tissue accumulation and turnover, the N- and C-isotope signatures of fish approach a representative stream value within 5–6 weeks after stocking. Of the different isotopic systems considered, our data indicate that the Sr signal is the most stable in fish over time, followed by N and then C. Differences among these isotopic systems arise from differences in their sources, the extent of biological fractionation, and their allocation and quantification in tissues ranging from highly labile (e.g., muscle) to highly conservative (e.g., otoliths).

The Sr isotope values are likely to be the most stable for several reasons. First, the $^{87}Sr/^{86}Sr$ ratio in stream water arises from bedrock geology (Kennedy et al. 2000). Second, there is no biological fractionation of Sr isotopes and the $^{87}Sr/^{86}Sr$ ratios in fish tissues closely match that of stream water (Kennedy et al. 2000). Third, time-specific Sr signatures are stored in otoliths and are not altered by tissue turn-

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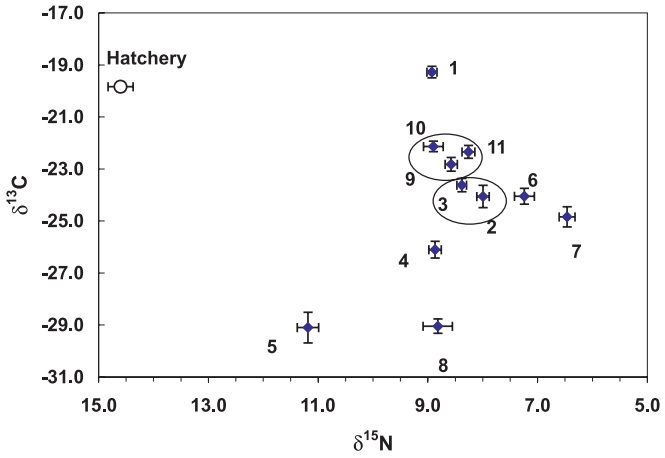
Table 2. Discrimination of fish from different field sites based on differences in their stable-isotope signatures.

Site	Reference No.[a]	n[b]	$\delta^{15}N$ group[c]	$\delta^{13}C$ group[c]	$\delta^{15}N$ and $\delta^{13}C$ group[c]	Individual reclassification[d]	$^{87}Sr/^{86}Sr$ group[e]
Marlboro	1	22/19	1	1	1	17 of 19	1
Tweed	2	26/23	1	2	2	2 of 23	2
Utley	3	22/19	1	2	2	0 of 19	3
White River	4	8/8	1	3	3	8 of 8	4
First Branch	5	6/6	2	4	4	6 of 6	5
West Branch	6	20/6	3	2	5	1 of 6	6
Bingo Branch	7	15/6	4	2	6	4 of 6	6
Third Branch	8	5/5	1	4	7	5 of 5	7
Bethel Gilead	9	32/32	1	5	8	7 of 32	7
Flood	10	12/12	1	5	8	9 of 12	8
Hancock	11	31/31	1	5	8	15 of 31	9
Total[f]	11 sites	199/167	4 distinct groups	5 distinct groups	8 distinct groups	74 of 167 (44.3%)	9 distinct groups

[a] Reference numbers refers to sites shown in Figs. 1 and 4.
[b] n denotes the number of fish from each site for both N and C (indicated as N/C). In all cases where N and C values were obtained, the analysis was performed jointly on the same individuals; however, in some cases only N was analyzed
[c] Differences in the isotopic signatures of fish from different sites (pooled within a site over all sampling dates) are denoted by different numbers within each column (based on Fisher's least significant difference multiple comparison as described in the text).
[d] Individual reclassifications are based on the numbers of individual for which group membership was correctly predicted using the results of a linear discriminant function analysis for N and C.
[e] Sr groupings are included for comparison and data are from Kennedy et al. (2000).
[f] The number of sites that could be statistically distinguished by different isotopic signatures.

Fig. 4. Discrimination between the 11 populations of salmon in this study, based on a combination of N- and C-isotope ratios. The open symbol represents the initial hatchery value. When N and C isotopes were used together, discrimination of populations was more than twice that of either method on its own. Two groupings of sites that could not be distinguished on the basis of their coupled isotopic signatures are circled.



over. Recent work shows that time-specific Sr signatures are recorded in otoliths and can be measured with high precision to reconstruct the movements of fish (Kennedy et al. 2002). Constraints on the successful use of this isotope are the spatial heterogeneity of the underlying geology and the relatively high labor intensity of analyzing Sr isotopes.

In contrast to Sr, N undergoes mass-dependent fractionation (≈3.5‰) at each trophic level (Deniro and Epstein 1978; Minagawa and Wada 1984; Robinson 2001). Hence, the $\delta^{15}N$ value for an individual at any point in time is based upon the trophic level from which that organism is feeding, which can change within or between seasons (Olson and Young 2003). N-isotope signatures were relatively stable in the fish from our six sites across the entire summer growing season. Despite fairly large increases in biomass, salmon in these streams appear to forage at the same trophic position throughout their first summer, which is consistent with our earlier work on the composition of the diet of juvenile salmon from these sites (Folt and Parrish 1994; Nislow et al. 1999). Hence, the differences in $\delta^{15}N$ values that we measured among sites appear to be more strongly driven by exogenous factors, such as differences in land use, which can create large differences in the $\delta^{15}N$ signal of stream water (Harrington et al. 1998), than by site-specific trophic differences or fish movements.

The C-isotope signature of fish changed the most over the course of the growing season. Based upon the quickness with which the N-isotope signal stabilizes, we believe the seasonal changes in C isotopes to reflect changes in the environment rather than a more gradual attainment of an equilibrial stream signature. These changes likely reflect either a change in the relative contribution of autochthonous and allochthonous C sources to the stream food web in particular locations (Rounick et al. 1982; France 1995*a*) or a seasonal change in the dissolved inorganic C profiles over algal substrates (Finlay et al. 1999). Neither stomach-content analysis (Folt and Parrish 1994) nor $\delta^{15}N$ values (this study) support a trophic shift during this period. A number of studies have used differences in the natural abundance of $\delta^{13}C$ to determine the source of C to the food web, and have shown that as this source varies, the signal in the consumers will also vary (Hamilton et al. 1992; Peterson et al. 1993; Doucett et al. 1996). The rate at which the tissue isotopic signature responds to changes in environmental input depends on the turnover rate in the tissue. Turnover and isotopic equilibration can take from days to weeks in muscle

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tissues (Hobson and Clark 1993; Herzka and Holt 2000). In this study, we investigated $\delta^{13}C$ signatures in the muscle tissue of rapidly growing salmon. Tissue turnover coupled with changes in the primary C source in some streams over time could easily explain the seasonal changes that we measured in $\delta^{13}C$. However, as in the case of $\delta^{15}N$, among-site differences in $\delta^{13}C$ were generally greater than the differences occurring within a site over the growing season, which is the period in which we are primarily interested. Consequently, $\delta^{13}C$ and $\delta^{15}N$ signatures provided short-term markers for salmon habitats over the summer growing season and would provide site-specific otolith signatures where lethal sampling was possible.

Nonlethal sampling

By investigating isotope ratios in the scales of the juvenile salmon, we tested the feasibility of nonlethal sampling of field-caught animals. We found a reliable correspondence between the $\delta^{15}N$ and $\delta^{13}C$ signature in scales and that in muscle tissue, which suggests that scales can provide a nonlethal marker for isotopic signatures in fish over short time scales. In the case of N, the slope of the line falls very close to the 1:1 line, suggesting that scale turnover is complete and in equilibrium with the relatively rapid exchange in muscle tissue. For C, an offset between the tissue and scale signatures is consistent with some residual hatchery signature being stored in the scales even after tissues have exchanged with the environment.

Our work with Sr isotopes in scales suggests that they also closely track the isotopic signature of their surrounding environment (Kennedy et al. 2000). Similarly, Wells et al. (2000*a*, 2000*b*) found that the trace elements (Sr, Cd, and Ba) in scales were incorporated in concentrations that were in relatively constant proportion to concentrations in the ambient water. Both of these studies analyzed only bulk scale material. Because the C signature from the hatchery appears to be retained in the scales of 3-month-old fish, scale annuli potentially serve as a time-specific repository for past signatures in a similar fashion to otoliths (Campana and Thorrold 2001; Kennedy et al. 2002). Thus, for applications that require the tracking of individual fish over long time periods (e.g., tracing the natal history in a migrating adult), $\delta^{13}C$ signatures or trace elements found in measurable concentrations in the annuli of scales may serve as markers. Future studies must address the time-specific storage of these signatures in scales to determine their relative success at providing reliable geochemical markers over the lifetime of fish.

Stock discrimination

All three isotopic systems successfully differentiated salmon, but they varied considerably in their discriminatory power. The $\delta^{15}N$ values of resident salmon differentiated approximately 33% of the randomly chosen salmon rearing streams. N isotopes are likely to be most valuable for differentiating fish that were reared in landscapes with varying degrees of agricultural impacts (as described in Harrington et al. 1998; McKinney et al. 2001). The sites used in this study varied only slightly with respect to land use, and no site was greatly impacted by agriculture (e.g., >15% by area). Therefore, our estimate of site discrimination is likely to be conservative when compared with its application in a heterogeneous landscape with a broad range of land uses represented.

In comparison, $\delta^{13}C$ differentiated 45% of the sites. These site-to-site differences likely arise from differences in the C sources of the food web (i.e., benthic algae versus allochthonous plant material), the concentration of dissolved inorganic C available to algae, the prevalence of C_3 and C_4 plants in the watersheds, and the geologic contribution to the isotopic composition. When applied randomly across the landscape (as in this study), the discriminatory power of C isotopes was good. Like N isotopes, C isotopes could also be selectively used to distinguish between fish from regions with known differences in terrestrial to autochthonous inputs. However, unlike N isotopes, C isotopes have been shown to vary over relatively small spatial scales (e.g., 10 to 100s of metres) (Finlay et al. 1999) and could be used to distinguish fish movements over different spatial scales (Folt et al. 1998).

Our overall success in discriminating between fish from different sites was greatest using Sr isotopes. This was true in part because the geology of this region is highly variable and produces a mosaic of $^{87}Sr/^{86}Sr$ ratios across the landscape (Kennedy et al. 2000). In situations where this is likely to be true, $^{87}Sr/^{86}Sr$ ratios are a powerful discriminatory tool. However, in combination, $\delta^{15}N$ and $\delta^{13}C$ also differentiated 73% of the sites, which approached the success of the $^{87}Sr/^{86}Sr$ ratio alone (83%). This result is particularly important because discrimination of fish stocks may be an important issue where homogeneous bedrock precludes the use of Sr isotopes. Additionally, considerably more laboratories are equipped to measure $\delta^{15}N$ and $\delta^{13}C$ than $^{87}Sr/^{86}Sr$. Hence, isotopic signatures of N and C may be particularly effective markers for juvenile salmon rearing locations.

Although more work is needed to establish the extent to which N and C signatures are preserved over long periods of time, our results suggest that these isotopic systems can be very useful in tracking individuals within at least one season of growth over multiple spatial scales. Examples of promising short-term applications include (*i*) following the dispersal of individuals both between sites and within seasons and (*ii*) identifying the tributary origins of outmigrating salmon smolts or mixed populations of other fish species. Adding other isotopes (such as O or H) to develop a multiple-isotope signature will augment our ability to identify rearing or natal origins of dispersing animals without the cost and biological damage associated with tagging or other invasive measures.

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